

P112**OA synovium contains subpopulations of mesenchymal progenitor cells with osteogenic and chondrogenic differentiation capacity**S. Fickert¹, J. Fiedler², R. Brenner³;¹Department Of Orthopaedic Surgery, University Hospital of Mannheim, Faculty of Medicine of the University of Heidelberg, Mannheim, Germany, ²Division Of Biochemistry Of Joint And Connective Tissue Diseases, University of Ulm, Ulm, Germany, ³Division Of Biochemistry Of Joint And Connective Tissue Diseases, university of Ulm, ulm, Germany**Purpose:** Identification, quantification and isolation of subpopulations with characteristics of mesenchymal progenitor cells (MPC) from the synovial membrane (SM) from patients with osteoarthritis (OA).**Methods and Materials:** Cells from the SM of patients with end stage OA who underwent total knee joint replacement were enzymatically isolated. One aliquot was directly analyzed by fluorescence automated cell sorting (FACS) using various combinations of surface markers of bone marrow MPC (CD9, CD44, CD54, CD90, and CD166). Remaining cells were cultivated on plastic, expanded over several passages, analyzed by FACS again and tested for their osteo- and chondrogenic potential. The differentiation was analyzed by immuno-/histochemistry and by RT-PCR for the expression of lineage related marker genes.**Results:** Using FACS analysis we could show that the relative proportion of subpopulations expressing triplicate combinations of CD9, CD44, CD54, CD90 and CD166 in the SM from OA patients varies between 3 and 10%. Upon cultivation their relative amount markedly increased to values between 24 and 48%. Within the heterogeneous cell populations it was possible to induce osteogenic and chondrogenic differentiation. Initial sorting for CD9/CD90/CD166 triplicate positive cells proved that this subpopulation contains cells with multipotency for mesenchymal differentiation and thus characteristics of MPC.**Conclusions:** Our results show that SM from OA patients contains cells that express typical combinations of MPC surface markers and have the potency of osteogenic and chondrogenic differentiation. Their relative enrichment during in vitro cultivation and the possibility of cell sorting to get more homogenous populations offer interesting perspectives for possible future therapeutic applications.**P113****Chemokines recruit human mesenchymal progenitors and are candidates for regenerative Tissue Engineering approaches**K. Neumann¹, M. Endres², J. Ringe³, M. Sittlinger³, C. Kaps⁴;¹TransTissueTechnologies GmbH, Berlin, Germany, ²R&D, TransTissueTechnologies GmbH, Berlin, Germany, ³Tissue Engineering Laboratory, Charité-University Medicine Berlin, Berlin, Germany, ⁴TransTissue Technologies GmbH, Berlin, Germany**Purpose:** The aim of this study was to analyze the potential of chemokines to attract mesenchymal progenitor cells (MPC) derived from bone marrow. Chemokines are attractive candidates for cell free Tissue Engineering approaches, thus guiding progenitors to the defect site.**Methods and Materials:** Human MPC were isolated as well as cultivated under standard conditions and characterized using FACS technique. The chemokine receptor profile of MPC was determined by real time gene expression analysis and immunohistochemistry. Recruitment of MPC by selected candidates was examined using the ChemoTx[®] System (Neuroprobe), in different concentrations (1-1000M) in each essay.**Results:** Human MPC showed a robust pattern of typical cell surface antigens like e.g. SH-2, SH-3 and CD90 and expressed a variety of chemokine receptors. Amongst others, MPC expressed the chemokine receptors CXCR1, CXCR2 and CCR2. These receptors bind to the chemokine ligands SDF-1, IL-8 and MCP-1. 96 well chemotaxis assays demonstrated that SDF-1 and IL-8 recruited MPC dose dependently, whereas MCP-1 did not recruit at all.**Conclusions:** Chemokines recruit MPC and therefore are promising candidates for cell free Tissue Engineering approaches, guiding multipotential progenitors to sites of injured tissue.**P114****Effect of three-dimensional expansion and cell seeding density on the cartilage-forming capacity of human articular chondrocytes in type II collagen sponges.**S. Francioli¹, C. Candrian¹, K. Martin², M. Heberer³, I. Martin³, A. Barbero⁴;¹Department Of Surgery And Of Research, University-Hospital, Basel, Switzerland, ²Division Biomaterials, Geistlich Pharma AG, Wolhusen, Switzerland, ³Department Of Surgery And Of Research, University Hospital Basel, Basel, Switzerland, ⁴Department Of Surgergergy And Research, University Hospital Basel, Basel, Switzerland**Purpose:** We investigated whether the quality of cartilaginous tissues generated in vitro by human articular chondrocytes (HAC) on collagen-II sponges can be enhanced by direct expansion of HAC on the biomaterial, as compared to standard monolayer on plastic, or by increasing cell seeding density.**Methods and Materials:** HAC were isolated from the cartilage biopsies of three different patients. Cell constructs were generated by: HAC expansion directly on collagen-II sponges (Chondrocell, Geistlich, CH) for 4 doublings (expansion time: 9days; density yielded: 35x10⁶cells/cm³) (group 1); HAC expansion in monolayer for 4 doublings (expansion time: 6days) and then seeding on the sponges at 35x10⁶cells/cm³ (group 2); HAC expansion in monolayer for 8 doublings (expansion time: 12days) and then seeding on the sponges at 35x10⁶ (group 3) or 70x10⁶cells/cm³ (group 4). Constructs were then cultured for 4weeks in medium promoting chondrogenesis. The generated tissues were assessed histologically (Safranin-O) and biochemically (glycosaminoglycans -GAG- and DNA).**Results:** HAC expanded in collagen-II sponges (group 1) or monolayer (group 2) for 4 doublings generated tissue with fibroblastic appearance and low GAG/DNA. Also HAC expanded in monolayer for 8 doublings generated tissue with poor quality when seeded at 35x10⁶cells/cm³ (group 3). However, when seeded at 70x10⁶cells/cm³ (group 4), HAC generated hyaline-like cartilaginous tissues intensely stained for GAG and containing 2.2-fold more GAG/DNA.**Conclusions:** High cell seeding density is a crucial factor for chondrogenesis of HAC in collagen-II sponges. Direct expansion on collagen-II sponges did not enhance the quality of the resulting cartilaginous tissues possibly because it did not allow to reach a sufficient cell density.**P115****IGF-1 gene-supplemented type II collagen scaffolds for mesenchymal stem cell-driven chondrogenesis in vitro**T. Gotterbarm¹, J. Niska², R.M. Capito³, S.M. Vickers⁴, M. Spector⁵;¹Department Of Orthopaedics, University of Heidelberg, Heidelberg, Germany, ²Va Boston Healthcare System, Harvard Medical School, Boston, MA, United States of America, ³Materials Science & Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States of America, ⁴Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, ⁵Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, United States of America**Purpose:** The purpose of this study was to develop an IGF-1 gene-supplemented type II collagen scaffold to stimulate stem cell-driven chondrogenesis in vitro.**Methods and Materials:** Highly porous scaffolds were fabricated by ramp-freeze-drying and dehydrothermal treatment of a type II collagen slurry (Geistlich Biomaterials, Wolhusen, Switzerland). IGF-1 plasmid DNA was complexed to a lipid-mediated transfection reagent and incorporated in the scaffolds. Bone marrow-derived mesenchymal stem cells were isolated from six Spanish goats, subcultured separately (to P2) and seeded onto the collagen sponges (4 million). Scaffolds with or without pIGF-1 were cultured for 14 days in serum-free chondrogenic media containing either rhTGF- β 1 (10ng/ml) or with additional rhIGF-1 (100ng/ml). At 3 and 14 days DNA and GAG content was biochemically assessed and the scaffold diameter was measured (n=6). Scaffolds were stained for hematoxylin/eosin, and Safranin-O/Fast green for GAG.**Results:** All scaffolds showed a comparable amount of cell-mediated contraction. At 3 days a significantly lower amount of DNA was found in gene-activated scaffolds (p<0.04), perhaps related to impaired cell adhesion. After 14 days of culture all groups displayed a significant increase of GAG deposition. pIGF-1 supplementation exhibited significantly higher values of GAG/DNA compared to controls. Histochemical staining with Safranin-O/Fast Green was consistent with the biochemical data.**Conclusions:** Our data suggest that transient incorporation of IGF-1 plasmid DNA into type II collagen scaffolds stimulates stem cell-driven chondrogenesis. Gene-supplemented scaffolds may be of value as implants to improve the outcome of selected cartilage repair procedures.